

CHAPTER 13

Potential for Biological Control in the Management of Cassava Pests*

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Biological Control

Biological control, in the ecological sense, as a phase of natural control, may be defined as the regulation of the population density of a pest organism by natural enemies (parasites, parasitoids, predators, or pathogens) at a level that would be equal or greater than would have been reached by means of another alternative. Applied biological control supposes professional activity or human manipulation that promotes the effectiveness of natural enemies (DeBach 1977).

In a broad sense, biological control may also be defined as mortality or suppression of pest organisms by any biotic factor. In this wider sense, it is the direct action of parasites, parasitoids, predators, and pathogens (i.e., natural enemies) and of competition with other species for natural resources (i.e., antagonists) that regulate an organism's population density to a level lower than it would have been in the absence of that control. It does not include plant resistance, interference with the pest by semiochemicals (e.g., pheromones, allomones, kairomones, and synomones), genetic engineering of the pest, natural chemical extracts, or mechanical control by humans. It does include the manipulation of natural enemies and antagonists through, for example, importation, mass-rearing, and release (Cave 1995a).

Research on biological control includes baseline surveys of any application of the method. These do not necessarily report immediately useful results or

direct methods for using, manipulating, or conserving natural enemies. In the first phases, the fundamental aspects studied are taxonomy, biology, physiology, genetics, ecology, demography, behavior, and nutrition of pests and their enemies (DeBach 1975).

If necessary, a pest and its enemies are identified by a specialist, as the organism's name and classification are key to all existing knowledge on it, and thus understanding and controlling it if it is pest, or using it if it is a natural enemy (Cave 1995b).

Natural Enemies

In biological control, various natural enemies participate, for example, parasites, parasitoids, predators, and microorganisms. These organisms must be able to respond quickly to the pest's population dynamics, so that proportionately more natural enemies are present as the pest population increases. This characterizes the theoretically ideal natural enemy, as well as certain biological and ecological criteria (Cave 1995c).

Predators

Predators are carnivorous organisms that, in either immature or adult state, actively seek and capture numerous prey, consuming them either partially or totally. Perhaps half of all insects and mites are predators. As they are so numerous, determining the most effective predators is difficult. They are classified as either generalists or specialists, according to eating habits and behavior. The principal arthropod predators belong to the following orders: Odonata, Orthoptera, Dermaptera, Hemiptera, Neuroptera, Coleoptera, Diptera, Hymenoptera, Araneae, and Acari. Families that stand out are Mantidae, Labiduridae, Pentatomidae, Chrysopidae, Carabidae, Staphylinidae, Coccinellidae, Elateridae, Cecidomyiidae, Syrphidae, and Phytoseiidae (Banegas and Cave 1995).

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Parasites

A parasite is an organism that lives at the expense of another organism—the host (Australian Museum 2005). In general parasites share the following features:

- Parasites are usually smaller than their host. Parasites use both invertebrate and vertebrate hosts.
- Adult parasites may live on the host (e.g., lice), in the host (e.g., tapeworms) or feed on a host occasionally (e.g., mosquitoes).
- Parasites generally do not kill the host but may harm the host indirectly by spreading pathogens. This may affect the host's behavior, metabolism or its reproductive activity.
- Many parasites have hooks, claws or suckers to attach to their host. Generally parasites have either a sucker (e.g., leeches) or piercing and sucking type mouthparts (e.g., fleas) for feeding.
- Both adults and young can be parasitic. In some cases the young are parasites but the adult is not.

Parasitoids

A parasitoid is an organism that has young that develop on or within another organism (the host), feeding on a single host and killing it at the end of their cycle. The adult state lives free and is not parasitic. Among the characteristics that make parasitoids promising for biological control are:

- Specificity (e.g., Aphelinidae and Encyrtidae)
- Ease of breeding in large quantities (e.g., *Trichogramma* spp., *Cotesia flavipes*, *Encarsia formosa*, and *Telenomus remus*)
- The power of flight, which facilitates dispersion
- High fertility, short generational time, and evolutionary rates that are comparable with those of the pests

Parasitoid species can be found in five of the insect orders, but most are in two: either the Hymenoptera or Diptera (Díaz and Hanson 1995). Important families include Aphelinidae, Platygasteridae, Eulophidae, and Encyrtidae.

Entomopathogens

As their name says, entomopathogens cause diseases in insects, and are grouped as microbiological

controllers. According to Castillo et al. (1995), the expression “microbiological control” refers to the use of microorganisms (which, in the broad sense, includes nematodes) to control pests.

Recently, the use of microorganisms to effectively control insect pests in different crops has increased, most likely as a result of the discovery and development of new species and strains of entomopathogens (Lacey and Brooks 1997). Insects associate with microorganisms in diverse ways such as symbiosis, mutualism, and parasitism. Mutualism abounds among insects; an example is the association of protozoa with termites, although the former are not pathogenic to the host insects. In contrast, entomopathogens cause infections, parasitism, or toxemia in insects (Lacey and Brooks 1997).

Five principal groups of microbiological agents exist: viruses, fungi, nematodes, bacteria, and protozoa.

Viruses. Entomopathogenic viruses are infectious entities whose genome, constituting nucleic acid, DNA, or RNA, is replicated in host tissues. A major viral family for insect control is the Baculoviridae. Baculoviruses contaminate the insect through the oral pathway. Normally, virions (infective units of viruses) are found on plant leaves and stems, and are ingested by the insect as it eats. The first cells to be affected are the epithelial ones of the intestine. The virus then attacks other tissues such as fatty bodies, intestinal epidermis, hemocytes, trachea, and silk glands. Infected larvae become lethargic, stop eating, and finally become paralyzed. Dead insects become the most important sources of inoculum for maintaining the epizootic (Castillo et al. 1995).

Fungi. Entomopathogenic fungi kill the host relatively quickly by penetrating and proliferating within its body. The insect dies as the fungus either deprives it of soluble nutrients from the hemolymph, invade or digest its tissues, or releases toxins that poison it. Not all fungi associated with insects are pathogenic. Some pathogens are obligate, but most are facultative. Saprophytic and other symbiotic fungi also exist (Ferron 1985).

More than 700 species of entomopathogenic fungi exist, distributed across different taxonomic groups, and all with potential for use in regulating insects (Hajek and St Leger 1994). The most widely accepted classification system of fungi was proposed by Ainsworth in 1973 (cited by Tanada and Kaya 1993). It

separates fungi into two divisions: Myxomycota, which are plasmodial (i.e., asexual, pseudopodial, and producing masses of multinucleate protoplasm that resemble amebas); and Eumycota, which are nonplasmodial and frequently mycelial in form. Entomopathogenic fungi are found in the division Eumycota and in the following subdivisions:

- Mastigomycotina: mobile cells or zoospores; perfect stage as oospores
- Zygomycotina: cells not mobile; perfect stage as zygospores
- Ascomycotina: perfect stage as ascospores
- Basidiomycotina: perfect stage as basidiospores
- Deuteromycotina: cells not mobile; no perfect stage

Most entomopathogenic fungi are found in the subdivisions Zygomycotina, class Zygomycetes, order Entomophthorales (e.g., *Neozygites* genus); and Deuteromycotina, class Hyphomycetes, order Moniliales (includes the genera *Aspergillus*, *Beauveria*, *Fusarium*, *Hirsutella*, *Metarhizium*, *Paecilomyces*, and *Verticillium*).

Researchers of entomopathogenic fungi have carried out studies that indicate an apparently logical way to demonstrate the virulence or pathogenicity of these fungi in different insects. Thus, they can enter in an integrated pest management (IPM) program (Sánchez 1996).

Nematodes. The phylum Nematoda is, after Arthropoda, the most diverse of the animal kingdom. They can be found in a wide variety of habitats. They are round worms that lack respiratory and circulatory systems. The insect-nematode association ranges from accidental to obligate and from commensal to parasitic (Stock 1998).

Nematodes of the families Steinernematidae and Heterorhabditidae are obligate parasites of a broad range of insect species. In each family, only one genus exists, respectively, the *Steinernema* and *Heterorhabditis*. Their members live in mutualism with the bacteria *Xenorhabdus* sp. and *Photorhabdus* sp., respectively (Sáenz 1999).

These bacteria, which are lethal for their insect hosts, make the nematodes an adequate organism for biological control. The infective state is the juvenile of the third nymphal stage (IJ3), which either enters through natural apertures such as the mouth, anus, or

spiracles, or penetrates the insect's cuticle to reach the hemocoel, where it releases the symbiont. The bacterium proliferates and produces enzymes (lipases and proteases) that degrade the host's tissues. Together, the nematodes and bacteria kill the insect within 48 h. The juveniles then consume the bacteria and the insect's degraded tissues.

Nematode development is favored by the bacterium producing antibiotics, preventing the proliferation of secondary pollutants. Between one and three generations of the nematode are produced in the host insect, depending on its size. All individuals of *Steinernema* are sexually differentiated. In *Heterorhabditis*, however, those of the first generation are hermaphrodite and those of the second are differentiated.

The bacteria *Xenorhabdus* and *Photorhabdus* are Gram-negative bacilli of the family Enterobacteriaceae (Akhrust and Boemare 1990). In *in vitro* culture, they present two phases:

- P1, where they live in infective nematodes of IJ3 form, producing antibiotics and flagella
- P2, where they live in cadavers of old insects or nematodes

The bacterium-nematode association is mutualistic: the bacterium cannot survive alone in the soil and the nematode cannot develop well without the bacterium. Through mutual assistance, they evade the host's immune response, thus guaranteeing the survival of both. However, more research is needed on this mechanism. Meanwhile, the pathogenic potential of the nematode-bacterium complex has been used for the biological control of several pests (Stock 1998).

Bacteria. Bacteria are found in all dead insects, but only some are the primary cause of mortality; others sometimes cause mild infections. The bacteria enter the insect through its food and remain confined by the intestine's peritrophic membrane. They cause general septicemia, but are not located in any specific tissue. Little is known of the role that bacterial pathogens play in controlling the insect pest. Epizootics occur under reported conditions of high-density populations of the host but, under other circumstances, either occur rarely or are unrecognized.

These bacteria separate into facultative and obligate pathogens. Entomopathogenic bacteria belonging to the genus *Bacillus* (e.g., *B. thuringiensis*,

B. cereus, *B. popilliae*, and *B. larvae*) are the most promising for controlling insect pests (Castillo et al. 1995).

Protozoa. Protozoa are considered important factors in the natural regulation of the population density of certain insects. However, they have not been much applied as microbial agents, as entomophilic species already cause chronic or debilitating infections in a wide range of hosts (Castillo et al. 1995).

Biological Control of Cassava Pests

The most common natural enemies of cassava pests belong to four groups: parasites, parasitoids, predators, and pathogens. Of these, entomopathogenic fungi, nematodes, and viruses stand out as being the most studied. At CIAT, a list of natural enemies was compiled, which included 76 parasites/parasitoids, 138 predators, and 38 pathogens (Table 13-1). Possibly, more species are still unreported.

Table 13-2 shows some of the major natural enemies that control the cassava pests. Below are discussed significant pests: cassava green mite, cassava mealybug, cassava subterranean burrower bug, whitefly, cassava hornworm, white grubs, stemborers, and lace bugs.

Cassava green mite

The cassava green mite (CGM), *Mononychellus tanajoa* (syn.: *Mononychellus progresivus*), is probably native to Northeast Brazil, where it was reported for the first time in 1938. The indigenous people knew its characteristic symptom of damaged young leaves and meristems, calling it *tanajoa* or "plant disease or problem" (Bellotti and Schoonhoven 1978; Bellotti et al. 1999). In the 1970s, *M. tanajoa* was introduced accidentally to the African continent, first appearing in Uganda (Nyiira 1972). This pest spread throughout the African cassava belt within 10 years, perhaps through the exchange of planting material (Yaninek and Herren 1988). This mite is currently the principal cassava pest in Africa, causing yield losses between 13% and 80% (Herren and Neuenschwander 1991).

To develop a biological control program to combat CGM—a pest of great importance in the subhumid areas of Africa and Brazil—studies were conducted on the taxonomy and geographical distribution of predator mites of the family Phytoseiidae in the cassava crop (Bellotti et al. 1983b). Geographical priority was assigned to the exploration of natural enemies based

Table 13-1. Natural enemies (no.) of cassava pests.

Pest	Biocontrollers (Enemy type)		
	Parasites/ Parasitoids	Predators	Pathogens
Mites			
<i>Mononychellus tanajoa</i>		60	2
<i>Tetranychus urticae</i>			
Hornworm	18	15	15
Whitefly	17	5	6
Lace bug			1
Mealybug	25	46	2
Fruit fly	3		
Stemborers			
<i>Chilomima clarkei</i>	5	2	5
<i>Lagochirus</i> sp.	2		
Scale insects			
<i>Aonidomytilus albus</i>	2	9	2
<i>Saissetia miranda</i>	2		
Cydnidae		1	2
Gall fly	2		1
White grubs			2
Total	76	138	38

on agrometeorological homology of those regions in the Americas and Africa affected by CGM (Bellotti et al. 1983b; Yaninek and Bellotti 1987). Agrometeorological homology maps were prepared, based on the microregional classification of the cassava crop as proposed by Carter et al. (1992).

According to Braun et al. (1993), during explorations in the cassava-cropping areas of South America between 1983 and 1990, a total of 40 phytoseiid species were found in cassava and neighboring plants, living in association with the complex of phytophagous mite species. Maximum diversity was verified in Colombia. Of these 40 species, 18 were the most frequently found in the crop (CIAT 1990). Currently, CIAT has a database that stores records belonging to 2416 samples collected from different countries during various exploration periods. In all, 4300 records had been collected and identified by CIAT or international taxonomists. Of these specimens, the project conserves 2368 slides.

During the operation of the "CGM Biological Control" Project, 31 countries of the Americas and other continents were sampled. In Colombia, 1576 samples were recorded, meeting the project's objective.

Table 13-2. Major natural enemies of the most important cassava pests.

Pest	Type of natural enemies		
	Parasitoids	Predators	Entomopathogens
Cassava hornworm <i>Erinnyis ello</i>	<i>Trichogramma</i> sp. (E) ^a <i>Telenomus</i> sp. (E) <i>Sphingid</i> sp. (E) <i>Cotesia americana</i> (L) <i>Euplectrus</i> sp. (L) <i>Apanteles flaviventris</i> (L) <i>Drino</i> sp. (L) <i>Euphorocera</i> sp. (L) <i>Sarcodexia innota</i> (L) <i>Thysanomia</i> sp. (L) <i>Belusia</i> sp. (L) <i>Forcipomyia eriophora</i> (L)	<i>Chrysopa</i> sp. (E) <i>Podisus nigrispinus</i> (L) <i>P. obscura</i> (L) <i>Polistes canadensis</i> (L) <i>P. carnifex</i> (L) <i>P. erythrocephalus</i> (L) <i>P. versicolor</i> (L) <i>Zelus</i> sp. (L) <i>Polybia emaciate</i> (L) <i>P. sericea</i> (L) <i>Calosoma</i> sp. (L) Spiders (several species) (L)	<i>Bacillus thuringiensis</i> (L) <i>Baculovirus</i> of <i>E. ello</i> (L) <i>Metarhizium anisopliae</i> (L) <i>Beauveria bassiana</i> (L) <i>Paecilomyces</i> sp. (L) <i>Nomuraea rileyi</i> (L) <i>Cordyceps</i> sp. (P)
Cassava mealybug <i>Phenacoccus herreni</i>	<i>Apoanagyrus diversicornis</i> <i>Anagyrus insolitus</i> <i>Anagyrus</i> sp. ca. <i>putonophilus</i> <i>Epidinocarsis elegeri</i> <i>Prochiloneurus dactylopii</i> <i>Chartocerus</i> sp. <i>Acerophagus coccois</i>	<i>Ocyptamus</i> sp. <i>Symphorobius</i> sp. <i>Hyperaspis</i> sp. <i>Cleothera onerata</i> <i>Nephus</i> sp.	<i>Cladosporium</i> sp. <i>Neozygites fumosa</i>
<i>P. madeirensis</i>	<i>Eusemion</i> sp. <i>Signiphora</i> sp.	<i>Kalodiplosis coccidarum</i> <i>Curinus colombianus</i> <i>Cleothera onerata</i>	
<i>P. manihoti</i>	<i>Epidinocarsis lopezi</i> <i>Gyranusoidea</i> sp. <i>Parapyrus manihoti</i>	<i>Diomus</i> sp. <i>Exochomus</i> sp. <i>E. flaviventris</i> <i>Symphorobius maculipennis</i> <i>Hyperaspis raynevali</i> <i>H. aestimabilis</i> <i>Diomus hennesseyi</i>	
Cassava green mite <i>Mononychellus tanajoa</i>		Insects: <i>Stethorus</i> sp. <i>Oligota</i> sp. <i>Chrysopa</i> sp. Phytoseiidae mites: <i>Typhlodromalus manihoti</i> <i>T. aripo</i> <i>Neoseiulus idaeus</i>	<i>Hirsutella thompsonii</i> <i>Neozygites</i> sp.
Lace bug <i>Vatiga manihotae</i>		<i>Zelus nugax</i> (N-A)	
Whitefly complex <i>Aleurotrachelus socialis</i>	<i>Encarsia hispida</i> <i>E. bellottii</i> <i>Eretmocerus</i> spp. <i>Euderomphale</i> sp. nov. <i>Signiphora</i> sp.	<i>Chrysodina</i> sp. <i>Delphastus</i> sp. <i>Delphastus</i> sp. pos. <i>quinculus</i> <i>D. pusillus</i> <i>Chrysopa</i> sp.	<i>Fusarium</i> sp. <i>Verticillium lecanii</i> <i>Beauveria bassiana</i> <i>Metarhizium anisopliae</i> <i>Paecilomyces</i> sp. <i>Cladosporium</i> sp.
<i>Bemisia tuberculata</i>	<i>Eretmocerus</i> spp. <i>Encarsia pergandiella</i> <i>E. hispida</i> <i>Euderomphale</i> sp. nov. <i>Metaphycus</i> sp.		
<i>Trialeurodes variabilis</i>	<i>Encarsia pergandiella</i> <i>E. hispida</i> <i>Eretmocerus</i> sp.		

(Continued)

Table 13-2. (Continued.)

Pest	Type of natural enemies		
	Parasitoids	Predators	Entomopathogens
<i>Aleurodicus dispersus</i>	<i>Aleuroctonus vittatus</i> <i>Eretmocerus</i> spp.		
Other whiteflies:	<i>Encarsia sophia</i> <i>E. luteola</i> complex <i>E. strenua</i> complex <i>Amitus macgowni</i>		
Subterranean burrower bug <i>Cyrtomenus bergi</i>		<i>Nerthra</i> sp.	<i>Heterorhabditis bacteriophora</i> <i>Steinernema carpocapsae</i> <i>Metarhizium anisopliae</i> <i>Beauveria bassiana</i> <i>Paecilomyces lilacinus</i>
White grubs <i>Phyllophaga</i> spp.; <i>Anomala</i> spp.			<i>Heterorhabditis</i> spp. <i>Steinernema</i> spp. <i>Metarhizium anisopliae</i>
Stem borers <i>Chilomima clarkei</i> <i>Lagochirus</i> spp.		<i>Bracon</i> sp. <i>Apanteles</i> sp. <i>Brachymeria</i> sp.	<i>Bacillus thuringiensis</i> <i>Spicaria</i> sp. Virus (not ident.)

a. Developmental stage at which the cassava pest are attacked: E = egg; L = larva; N = Nymph; P = pupa; A = Adult.

The most sampled areas were in Colombia, Venezuela, Ecuador, and Brazil. About 87 species were found, of which 25 were new. On cassava, 66 phytoseiid species were collected, with 13 being the most common (Melo 2000).

Typhlodromalus manihoti was collected the most frequently, being found in more than 50% of the sampled fields, followed by *Neoseiulus idaeus*, *T. aripo*, *Galenromus annectens*, *Euseius concordis*, and *E. ho*. To control *M. tanajoa* in Africa, *T. aripo* and *N. idaeus* showed the most promise (Yaninek et al. 1991, 1993). Since 1984, numerous phytoseiids species have been sent from Colombia and Brazil to Africa.

Of the species that were mass released and established, none came from Colombia. However, three successful species were from Brazil: *T. manihoti*, *T. aripo*, and *N. idaeus* (Yaninek et al. 1991, 1993; Bellotti et al. 1999). *Typhlodromalus aripo* appeared to be the most promising as it dispersed rapidly to more than 14 countries. Field evaluations indicated that *T. aripo* reduces the CGM population by 35% to 60%, thus increasing fresh matter production by 30% to 37%.

Field experiments conducted in Colombia (Braun et al. 1989) demonstrated the importance of diversity of phytoseiid species for controlling the CGM. In Colombia, fresh and dried roots production was reduced by 33%

when natural enemies were eliminated. Neither did acaricide applications increase production, thus indicating the effectiveness of biological control.

The explorations also identified some predator insects of the CGM, especially the staphilinid *Oligota minuta* and the coccinellid *Stethorus* sp. *Oligota minuta* has been classified as the dominant predator of *M. tanajoa* populations. Research conducted at CIAT and in Uganda agrees that *Oligota* populations are located among the fifth and eighth leaves, that is, where the pest is found in highest numbers. One larva can consume from 49 to 70 mites and from 44 to 61 of their eggs. One adult consumes, over 7 to 16 days, between 97 to 142 eggs and adults.

The other insect predator, *Stethorus* sp., is found more in association with another pest: the spider mite *Tetranychus urticae*. In severe attacks in the field, 98% of predators were *Stethorus* sp. and only 2% were *Oligota* sp. (CIAT 1982).

These phytoseiids and predator insects are being intensively studied in the laboratory and field. So far, studies have shown that phytoseiid mites are more efficient than predator insects (Byrne et al. 1983), although laboratory and field studies have shown that the neuropteran predator *Chrysopa* sp., which consumes the pest at different stages, is also very effective.

Other natural enemies of the pest mites are pathogenic fungi belonging to the genera *Neozygites* (Zygomycetes: Entomophthorales) and *Hirsutella* (Hyphomycetes: Moniliales). The former is a pathogenic fungus that appears sporadically in Colombia and Northeast Brazil (*Neozygites* sp. cf. *floridana*) and causes as much as 100% mortality in CGM in 1–2 weeks (Delalibera et al. 1992). Some strains are specific to the *Mononychellus* genus (Moraes and Delalibera 1992).

This pathogen has been also found in Africa, but has never been observed as causing dramatic mortality in this pest (Yaninek et al. 1996). This suggests that isolates from Brazil are more virulent than those of Africa. Because the taxonomy of this genus is not well known and it is necessary to differentiate the African isolates from the candidates for release, molecular analysis of these has started. Results indicate that the isolates can be differentiated, although the technique needs to be standardized to measure genetic distance (Bohórquez 1995).

Hirsutella sp. was evaluated in Africa and shown to be very effective. Its potential is such that it could be used as a biological control agent (Odongo et al. 1988; Yaninek et al. 1996).

Cassava mealybug

One way to control mealybug pests (*Phenacoccus herreni* and *P. manihoti*) is to use natural enemies, finding them through explorations. The management of the cassava mealybug is an example of classical biological control (Herren and Neuenschwander 1991). A complex of mealybug species exists, as mentioned in previous chapters, including *P. herreni*, which is found in the Americas. Of its parasites, two species of *Anagyrus* (Encyrtidae) stand out: *A. insolitus* and *A. sp. ca. putonophilus*. Several parasitoids show a specialty or preference for *P. herreni*. Among those identified in northern South America are *Acerophagus coccois*, *Apoanagyrus diversicornis*, *Ap. elegeri*, *Anagyrus putonophilus*, *A. insolitus*, and *Aenasius vexans*. Three of these (*Ap. diversicornis*, *Ac. coccois*, and *Ae. vexans*) were identified as effective for controlling *P. herreni* (van Driesche et al. 1988, 1990).

Collaborative efforts by CIAT and EMBRAPA ensured that *Ap. diversicornis*, *Ac. coccois*, and *Ae. vexans* were exported from CIAT and released in Northeast Brazil, mainly in the states of Bahia and Pernambuco, during 1994 to 1996. Before introduction, EMBRAPA scientists had carried out field

surveys to measure damage and collect natural enemies. By the end of 1996, more than 35,000 individuals of the three parasitoid species had been released.

In Bahia, *Ap. diversicornis* had dispersed 130 km in 6 months, 234 km in 14 months, and 304 km in 21 months, after release. *Acerophagus coccois* had also established successfully, being recovered in high numbers at distances of almost 180 km from the release site 9 months later. *Aenasius vexans*, however, was constantly recaptured at its release site in Pernambuco, having dispersed only 40 km in 5 months (Bento et al. 1999). Comparative studies of the life cycles of the three parasitoids show that each could complete two cycles for one cycle of *P. herreni*, a favorable ratio in biological control.

Aenasius vexans and *Ap. diversicornis* show a marked preference for *P. herreni*, even though laboratory studies indicated that they also parasitize other species of mealybug (Bellotti et al. 1983a, 1994; Bertschy et al. 1997). *Acerophagus coccois* shows equal preference for either *P. herreni* or *P. madeirensis*. All three parasitoids are attracted by infestations of *P. herreni* (Bertschy et al. 1997). *Apoanagyrus diversicornis* prefers third-instar nymphs, while *Ac. coccois*, which is much smaller, parasitizes with equal frequency male cocoons, adult females, and second-instar nymphs. Oviposition by *Ap. diversicornis* causes 13% mortality among third-instar nymphs (van Driesche et al. 1990). *Aenasius vexans* prefers with equal frequency second and third instars and adult females (CIAT 1990).

Some field studies of natural populations of *Ap. diversicornis* and *Ac. coccois* determined the percentage of parasitism by using plant traps as hosts of *P. herreni* around the cassava crop (van Driesche et al. 1988). With the combined action of the two parasitoids, *P. herreni* mortality was estimated at 55% (van Driesche et al. 1990).

In 1980, the cassava mealybug found in the Americas and that in Africa were reported as comprising different species. One presented males, which led to the description of a new species, *P. herreni*. At the same time, *P. manihoti* was located in Paraguay by Bellotti (Herren and Neuenschwander 1991). In addition to the pest, they found 15 natural enemies, two of which were sent for release in Africa. These were a coccinellid predator, *Cleothera onerata*, which, however, had difficulties surviving the rainy seasons; and the other was the parasitoid

Epidinocarsis lopezi, which was released by air and proved to be more effective. Its establishment was achieved in 25 countries of the cassava belt. The mealybug is now under control in 90% of the region (Wigg 1994).

The predators reported as attacking the mealybug (Table 13-1) include *Cleothera onerata* (as mentioned above), *Symphherobius* sp., the dipteran *Ocyptamus* sp., *Hyperaspis* sp., *Nephus* sp., and *Diomus hennesei*. The only natural enemy of *P. manihoti* found in Zaire was the predator butterfly *Spalgis lemolea* (Bennett and Greathead 1978; Leuschner and Nwanze 1978).

Entomopathogenic fungi also have been found in association with the cassava mealybug, for example, *Cladosporium* sp. and *Neozygites fumosa* (CIAT 1990).

Cassava subterranean burrower bug

CIAT has carried out baseline surveys on the cassava subterranean burrower bug, *Cyrtomenus bergi* (Hemiptera: Cydnidae), studying such aspects as biology, behavior, population fluctuation, and host preference (Arias and Bellotti 1985). Trials have also carried out on chemical control and cropping with the legume *Crotalaria juncea* (Castaño et al. 1985). Insecticides are not recommended, not only because they are costly, but also because they destroy the natural enemies that control the populations of other cassava pests (Caicedo and Bellotti 1994).

Native nematodes have been found in association with *C. bergi* and are considered as an alternative to chemical and agronomic control. Eight sites around Manizales, Pereira, and Santander of Quilichao have been explored and, in all samples, nematodes were found. Geographical races of *Heterorhabditis bacteriophora* were identified in 37% of isolates recovered from both soil and dead burrower bugs in the field under various climatic and physicochemical soil conditions (Caicedo and Bellotti 1996).

In the interest of control, a search and identification of native isolates of entomopathogenic nematodes (EPNs) were carried out in 2003, collecting soil samples from Quindío, Risaralda, Caldas, and Cauca. To extract EPNs, traps comprising larvae of the insect *Galleria mellonella* (Lepidoptera: Pyralidae) were used. The nematode larvae's pathogenicity was then verified, following the Koch postulates. They were then multiplied, stored, and identified. From 284 soil

samples—300 g each—collected from 15 crops at 23 sites, 11 were positive for fungi, 13 for mites, and 17 for entomopathogenic and saprophagous nematodes. From the latter, 20 subsamples were selected according to their morphological characteristics and behavior containing only entomonematodes and they were sent to Germany for identification.

Using the PCR molecular technique, two samples from Cauca (in *Manihot esculenta*) and Risaralda (in *Inga* spp.), respectively, were identified as *Steinernema kraussei* Steiner (Rhabditida: Steinernematidae). This was the first report of these nematodes for Colombia (Melo et al. 2009).

Caicedo (1993) reported that, when she used an isolate of the *S. carpocapsae* strain All, the most susceptible stage of the *C. bergi* proved to be the adult, which presented a 60% parasitism for the entire evaluated doses (2000, 4000, 6000, 8000, and 10,000 EPNs/ml). Over time (2, 5, 8, and 10 days), the nematodes caused 100% parasitism, but mortality was always lower than parasitism. The best dosages for mortality were determined to be LD₅₀ of 193 EPNs/ml and LD₉₀ of 403 EPNs/ml. On evaluating native isolates found in the samples, the fifth state of the pest was the most susceptible, with 90% succumbing to the isolate from Cauca (SQC92) and 100% to that from Risaralda (LFR92). The next most susceptible state was the adult, with 85% and 100%, respectively, succumbing.

Although the nematodes were able to parasitize all states of the pest, 100% parasitism by isolate LFR92 was effective only at 702 EPNs/ml and by isolate SQC92 at 826 EPNs/ml. Mortality, using LD₅₀ and the same strains, needed 800 and 870 EPNs/ml, respectively (Barberena 1996). At the end of 2002, soil samples were collected from the same sites where the EPN strains were initially found. The *Heterorhabditis* sp. strain CIAT was found at La Colonia, Department of Risaralda. Moreover, native and introduced strains of *Steinernema* and *Heterorhabditis* were evaluated on stages 5 and adult of the pest (5000 EPNs/ml). Values of 100% parasitism and 22% mortality were obtained for isolate *Steinernema* sp. strain SNI (CENICAFE) of the adult pest.

Greenhouse experiments were carried out on *C. bergi* applying *S. carpocapsae*, *Steinernema* sp. strain SNI, and *Heterorhabditis* sp. strain HNI, using a concentration of 1000 EPNs/ml. Values for parasitism on the pest were 21%, 18%, and 18%, respectively,

with no mortality. At a higher concentration (25,000 EPNs/ml), parasitism was 55% for *S. carpocapsae* and 45% for *Heterorhabditis* sp. strain HNI, and mortality 29% and 9%, respectively. In another experiments, entomonematodes *S. riobravo*, *Steinernema* sp. strain SNI, and *Heterorhabditis* sp. strain CIAT were evaluated against the third pest stage, with higher concentration (100,000 EPNs/ml). Pest mortality was 33%, 28%, and 26%, respectively.

During this research, on dissecting the burrower bugs, melanization of the EPNs was observed. With the collaboration of the chemistry laboratory at the University of Caldas, phenoloxidase activity was detected. It is probably the pest's immune response to attack from EPNs, injecting them, whether dead or alive. This finding is considered sufficiently important to further study of this line of inquiry to understand how this humoral response, typical in Diptera, appears in this hemipteran (CIAT 2003).

Two EPNs—the native *S. feltiae* (strain sampled at Villapinzón) and the introduced *Heterorhabditis bacteriophora* strain E-Nema—were evaluated for their parasitism on six developmental stages of *C. bergi*. Results were 45.2% and 46.8% infection for *S. feltiae* and *H. bacteriophora*, respectively, on all developmental stages of the pest at applied doses. Despite the lack of statistical differences between strains, the trend was greater infectivity for the fourth instar (48.4%) and adult (46.9%). Isolates of *S. feltiae* induced a mortality rate of 21.4% and those of *H. bacteriophora* 20.0%. Despite the higher infection rate, *H. bacteriophora* nevertheless showed a lower mortality rate.

Commercial concentrations—at 1000 and 500 EPNs/ml of *S. feltiae* and *H. bacteriophora*, respectively—were applied to fifth instars and adults of the pest, and destructive evaluations were made 15 and 30 days after infection (dai). Only the adults were infected, at 93.9% with *S. feltiae* and 72.1% with *H. bacteriophora*, with no distinction of strain or evaluation time. For mortality, at 15 dai, *H. bacteriophora* accounted for 41.2%, and *S. feltiae* 8.6%. However, at 30 dai, they equalized at 62.7%. Dissection of pest individuals revealed melanized EPNs, probably because of *C. bergi*'s immunological response to the EPNs. At 30 dai, *S. feltiae* was shown to be more susceptible (37.5%) than *H. bacteriophora* (13.17%).

Considering the behavior of *S. feltiae* isolates, which was more affected by the pest's defense, the

nematode-bacterium complex probably had difficulty developing and thus needed more time to kill the insect. Or, this increased exposure enabled more nematodes to enter and overcome the host's defenses.

Other studies have been conducted to find the best methodology for mass-rearing nematodes, using *H. bacteriophora*, which is considered as promising for its high virulence, its capacity to search, and facility to reproduce (Gaugler and Kaya 1990). Results indicated that the best production was obtained by breeding *in vivo* and *in vitro*. Two races of this species were also evaluated for their capacity to parasitize the entire pest's developmental stage. The fifth stage proved to be the most susceptible. On increasing nematode dose, parasitism also increased (Barberena 1996).

Other entomopathogens used for controlling *C. bergi* are fungi. Bioassays were carried out in the laboratory and suspensions of conidia of the fungus *Beauveria bassiana* were evaluated, together with *Metarhizium anisopliae* and *Paecilomyces lilacinus* (Deuteromycotina: Hyphomycetes), combining three substrata and two inoculation methods. Immature stages of the pest are the most susceptible to *M. anisopliae*, which induces higher mortality rates than either *B. bassiana* or *P. lilacinus* (Sánchez and Bellotti 1997a).

Isolates of *M. anisopliae* from CIAT applied in the laboratory also showed mortality rates ranging from 45% to 60% (Jaramillo 2004). Results showed that *C. bergi* can be controlled by using a combination of *M. anisopliae* (1E+08 conidia/ml) and sublethal doses (30 ppm) of Imidacloprid over 25 dai. The mortality rate was >80%. These greenhouse results were better than those obtained when only the insecticide was applied at commercial doses (Table 13-3). This is therefore an important alternative for IPM programs in Colombia and other Latin American countries, as it would encourage farmers to reduce their use of highly toxic synthetic insecticides such as chlorpyrifos and carbofuran, which are heavily used in Colombia (Jaramillo 2004).

Research indicates the potential that entomopathogenic nematodes and fungi have for the biological control of *C. bergi*, with recent studies indicating one possible solution. However, such research has been conducted only in laboratories or greenhouses. Field studies must be carried out before acceptable technologies can be recommended (AC Bellotti 2002, pers. comm.).

Table 13.3. Mortality average corrected (CM) (%) (\pm SD) of *Cyrtomenus bergi* nymph treated with *Metarhizium anisopliae* ($1E+08$ conidia/ml) and two doses of Imidacloprid (300 y 30 ppm) alone and combined with *M. anisopliae*.

Treatment	Percentage MS (\pm SD)					
	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
<i>M. anisopliae</i> + Imidacloprid 30 ppm	9.2 \pm 3.6 ab	22.4 \pm 6.0 a*	34.9 \pm 4.0 a*	61.2 \pm 5.4 a*	79.4 \pm 3.5 a*	87.1 \pm 2.9 a*
<i>M. anisopliae</i>	6.7 \pm 2.2 ab	13.5 \pm 3.9 a*	20.4 \pm 4.0 ab*	43.5 \pm 8.1 ab*	58.0 \pm 6.8 b*	66.5 \pm 5.2 b*
Imidacloprid 300 ppm	6.7 \pm 2.6 ab	10.8 \pm 4.2 Ab	20.6 \pm 3.6 ab*	35.6 \pm 5.1 bc*	52.2 \pm 4.8 b*	52.7 \pm 5.6 bc*
Imidacloprid 30 ppm	7.6 \pm 1.8 a*	15.2 \pm 4.0 a*	15.2 \pm 4.0 b*	18.8 \pm 4.0 c*	25.1 \pm 5.6 c*	37.4 \pm 5.9 c*

MS = Mean square; SD = Standard deviation.

A potential predator of *C. bergi* is the *Nerthra* bug (Hemiptera: Gelastocoridae), which was observed in a peanut field (MP Hernández 2002, pers. comm.).

Whitefly

Recently, in Colombia, whiteflies have caused adverse effects in areas where cassava is cultivated. Given this situation and the ignorance of the roles that biological control agents play, a study was begun of the parasitoid species that associate with this insect and their distribution. The study was conducted in different regions of Colombia: Cauca, Valle del Cauca, and the Atlantic Coast (Table 13-4)

(CIAT 1995). Samples of whitefly were duly collected and processed in the laboratory, identifying and analyzing each species of both parasitoids and whitefly.

To date, various species of whitefly and their parasitoids have been found in different areas or sites, demonstrating the variability of parasitoids and their intrinsic relationship with any given whitefly species, or their presence as hyperparasitoids. The following whiteflies were identified as being predominant in the crop: *Aleurotrachelus socialis*, *Bemisia tuberculata*, *Trialeurodes* sp., and *Tetraleurodes* sp.

The parasitoids found in association with whiteflies were *Eretmocer* sp. (Aphelinidae); the *Encarsia*

Table 13-4. Whitefly species and their parasitoids collected from three geographical regions of Colombia.

Region	Whitefly species	Parasitoid species
Atlantic Coast	<i>Aleurotrachelus socialis</i>	<i>Encarsia</i> sp. <i>Eretmocer</i> sp.
	<i>Bemisia tuberculata</i>	<i>Encarsia</i> sp. <i>Eretmocer</i> sp. <i>Metaphycus</i> sp.
	<i>Trialeurodes</i> sp.	<i>Encarsia</i> sp.
	<i>Tetraleurodes</i> sp.	<i>Eretmocer</i> sp.
	<i>Aleurotrachelus socialis</i>	<i>Encarsia</i> sp. <i>Eretmocer</i> sp. <i>Bemisia tuberculata</i>
Valle del Cauca	<i>Aleurotrachelus socialis</i>	<i>Encarsia bellottii</i> <i>Eretmocer</i> sp. <i>Signiphora aleyrodis</i>
Cauca	<i>Bemisia tuberculata</i>	<i>Encarsia pergandiella</i> <i>Eretmocer</i> sp. <i>Euderomphale</i> sp. <i>Signiphora aleyrodis</i>
	<i>Trialeurodes</i> sp.	<i>Encarsia hispida</i> <i>Encarsia pergandiella</i> <i>Eretmocer</i> sp.

pergandiella species group (Aphelinidae); *E. hispida*, *E. bellottii*, *Metaphycus* sp. (Encyrtidae), and *Euderomphale* sp. (Eulophidae). *Signiphora aleyrodinis* (Signiphoridae) is a possible hyperparasitoid (Trujillo et al. 1999). Other parasitoids identified were *E. sophia*, the *E. luteola* species group, the *E. strenua* species group (with the last two forming a species complex), and *Amitus macgowni* (HE Trujillo 2002, pers. comm.).

The greatest wealth of parasitoid species in Colombia (mainly of the *Encarsia*, *Eretmocerus*, and *Amitus* genera) was most frequently associated with *A. socialis*, *B. tuberculata*, and *Trialeurodes variabilis* (Castillo 1996).

Aleurotrachelus socialis, *B. tuberculata*, and *T. variabilis* are the whitefly species that usually attack the cassava crop in Colombia. Temperatures and humidity were not related to populations of the three species, although *A. socialis* was found primarily in those sites where temperatures were about 35 °C. More than 10 species of microhymenopteran parasitoids—natural enemies associating with whitefly species—were collected and identified. Most were newly recorded for Colombia (Castillo 1996). Three were identified as *Encarsia hispida*, *E. pergandiella*, and *E. bellottii* (Evans and Castillo 1998). Only one *Eretmocerus* and one *Amitus* sp. (*A. macgowni*) were identified. Predominant species were *E. hispida*, *Amitus* sp., and *Eretmocerus* sp. The highest levels of parasitism on whiteflies *A. socialis*, *B. tuberculata*, and *T. variabilis* were 15.3%, 13.9%, and 12.1%, respectively, although rates varied with geographical region (Castillo 1996).

The species complex of parasitoids associated with each whitefly species was, to some extent, influenced by geographical area. In the Caribbean coast, *A. socialis* was more frequently parasitized by *Eretmocerus* spp. (67%), whereas in Cauca and Valle del Cauca, the *Encarsia* genus complex was more predominant. For example, in Valle del Cauca (1000 m above sea level), 99.6% of parasitism of *A. socialis* was by *Encarsia* spp. versus 0.4% by *Eretmocerus* spp. (Figure 13-1) (Trujillo et al. 1999). The most numerous complex of parasitoid species was associated with *B. tuberculata*.

Greenhouse studies (Ortega 1999) showed that *E. hispida* preferred parasitizing the third instar of *A. socialis* (75.3%) to the other instars, with rates being 15.6%, 44.7%, and 43.1% for the first, second, and fourth instars, respectively. Another results also indicated that the third instar of the whitefly is also

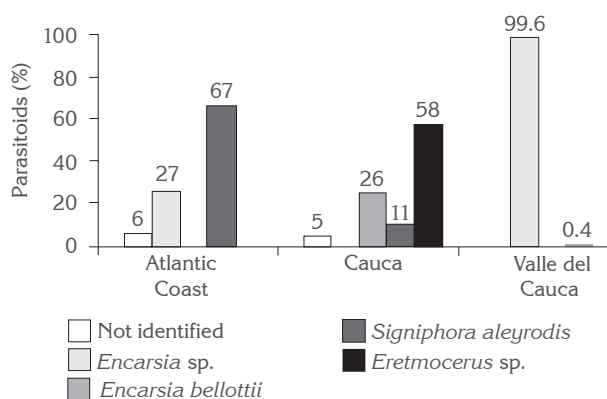


Figure 13-1. Parasitoid species collected on the whitefly *Aleurotrachelus socialis* from three areas of Colombia.

preferred by the parasitoid *E. hispida*, which showed an average parasitism rate of 21.1%, 35.2%, 46.4%, and 21.9% on the first through to the fourth instar, respectively. The highest parasitism rate was presented on the third instar. Evaluations were made 48, 72, 96, and 216 h after the parasitoids were released. The peak for parasitism occurred between 72 and 96 h, with 34.7% and 32.7%, respectively (Ortega 1999).

Although the parasitoid demonstrates facility to parasitize under controlled conditions, results under natural conditions may be less efficient. More research is needed in this area (CIAT 1999).

In Colombia, entomopathogenic fungi (*B. bassiana*, *Verticillium lecanii*, and *M. anisopliae*), recognized worldwide as whitefly pathogens, have been evaluated in the laboratory, but as yet have not been found parasitizing in the field. Laboratory results showed that, with *B. bassiana*, mortality rates were 28%, 55%, and 39% for nymphs of first, second, and third instars of *A. socialis*, respectively. The second instar was the most susceptible. *Beauveria bassiana* and *M. anisopliae* caused mortality rates of 18.1% and 18.8%, respectively, when applied in the morning, and 12.4% and 5.7% when applied in the afternoon (Sánchez and Bellotti 1997b).

Cassava hornworm

Several parasitic insects, predators, bacteria, fungi, and viruses make biological control of the cassava hornworm *Erinnyis ello*; also known as the ello sphinx moth, possible without having to resort to insecticidal applications that would otherwise break the balance existing between this pest and its natural enemies (Herrera 1999). More than 40 species of parasites,

predators, and pathogens of the pest's eggs, larvae, and pupae have been identified (CIAT 1989; Bellotti et al. 1999).

Eight species of microhymenopterans from the families Trichogrammatidae, Scelionidae, and Encyrtidae parasitize *E. ello* eggs. These include *Trichogramma minutum*, *Telenomus sphingis*, *T. dilophonotae*, *Ooencyrtus* sp., and *O. submetallicus* (CIAT 1989). Some *Trichogramma* and *Telenomus* species have been reported as parasitizing 94% to 99% of eggs (Bellotti and Schoonhoven 1978). The dipteran Tachinidae flies and hymenopteran Braconidae wasps, especially the *Cotesia* genus, also attack the pest (Bellotti et al. 1992, 1994).

The most common egg predators are the *Chrysoperla* spp. Other important predators, attacking larvae, include *Polistes* spp. (Hymenoptera: Vespidae), *Podisus* spp. (Hemiptera: Pentatomidae), and several species of spiders (Bellotti et al. 1992).

For microbial control, sprays of the bacterium *Bacillus thuringiensis*, in doses of 2 to 3 g of commercial product per liter of water, provide effective control. Control is more effective with first, second, and third instar larvae of the pest (Arias and Bellotti 1977; Herrera 1999).

The key to effective use of biological control agents is the ability to synchronize the release of a large number of predators or parasitoids during the pest's early developmental stages, preferably the egg or first to third instars. Parasitoids and predator efficiency is limited by poor functional response during short-term (15 days) hornworm outbreaks. Successful control requires the monitoring of populations in the field to detect immigrant adults or early instars. This can be done with black light lamps (type T20T12BLT) that trap adults in flight, or recognizing the presence of eggs or larvae (Braun et al. 1993). The difficulty of synchronizing mass releases of parasitoids and predators with peak populations of the pest suggests the need for an inexpensive, storable, biological pesticide.

A baculovirus has been identified as killing larvae, as being easy to manipulate, and inexpensive to store. This methodology was first implemented in commercial crops in Brazil, against populations of first-instar larvae. The result was almost complete control (Schmitt 1988). In Venezuela, the virus replaced insecticides in large plantations (7000 ha) where the hornworm is endemic. Control was 100% when

70 ml/ha were applied to larvae of first and second instars. The direct expense for storing, applying, processing, and collecting was US\$4/ha (CIAT 1995; Laberry 1997).

Entomopathogenic fungi also exist, although surveys showed that the number of insects affected by these in cassava crops was low, being found in only one of five areas evaluated. Under laboratory conditions, a strain of *B. bassiana* caused the highest mortality rate in *E. ello* (31.6% to 87.5%), with the third instar being the most susceptible. The fungus's action does not transmit from one generation to another. When two strains of *B. bassiana* and *M. anisopliae* are mixed and applied to third-instar larvae, the mortality rate was 90%. No antagonism was presented, with individual dead larvae showing typical symptomatology (Múnera et al. 1999).

In April 1979, during an outbreak of *E. ello* in the cassava-producing area of Quindío, Risaralda, and northern Valle del Cauca, pupae of this insect were collected, showing infection by a fungus of the *Cordyceps* genus (class Ascomycetes). In this same area, the pathogen had contained the attack by the pest's third generation. The fungus can be cultivated under laboratory conditions in oat-agar medium (CIAT 1989).

White grubs

Nematodes *Steinernema* sp. strain SNI, *Heterorhabditis* sp. strain HNI, and *Heterorhabditis* sp. strain CIAT were evaluated under the controlled laboratory conditions. On third-instar larvae of *Phyllophaga menetriesi*, penetration by the nematodes was 74.5%. The highest was for *Steinernema* sp. at 80.0%, compared with 52.9% for *Heterorhabditis* sp. strain CIAT. Overall, the mortality rate was 10.5%. Further experiments were carried out with three other strains of entomonematodes (SNI, HNI, and H-CIAT), using two concentrations (7000 and 13,000 EPNs/ml) and two periods of evaluation (5 and 10 days). *Heterorhabditis* strain HNI-13-5 induced the highest mortality rate at 31.6%, and strains HNI-13-10 and H-C-7-5 both induced a rate of 21%. Treatments with *Steinernema* strains SNI-13-5 and SNI-7-10 showed no mortality. That is, mortality rates from treatments with the highest percentages of penetration (*Steinernema* sp.) were lower than those of *Heterorhabditis* sp., which, with less parasitism, caused higher mortality.

Later tests with *Phyllophaga* sp. evaluated the infectivity and mortality produced by seven strains of

EPNs: *Steinernema riobravis* (Sr), *S. carpocapsae* strain All (Sc), *S. arenarium* (Sa), and *S. feltiae* (Sf); and *Heterorhabditis bacteriophora* strains Hb1 and Hb2, and *Heterorhabditis* sp. strain HNI. The concentration was 10,000 EPNs/ml. The highest values for infectivity occurred with the *Heterorhabditis* strains Hb2 (70.8%), HNI (74.0%), and Hb1 (77.1%), whereas *Steinernema* strains had the following rates: Sr at 12.5%, Sc at 13.5%, Sa at 17.7%, and Sf at 35.4%. The survival rate was higher for the *Steinernema* strains Sf (75.0%), Sa (92.7%), Sr (97.9%), and Sc (98.9%) than for the *Heterorhabditis* strains HNI (30.2%), Hb2 (35.4%), and Hb1 (40.6%).

Three species of white grubs were also evaluated (*Anomala* sp., *Phyllophaga* sp., and *P. menetriesi*) against three *Heterorhabditis* strains (*H. bacteriophora* Hb1 and Hb2, and *Heterorhabditis* sp. strain HNI) and two *Steinernema* species (Sr and Sc). The average rates of infection of *Phyllophaga* sp., *Anomala* sp., and *P. menetriesi* by the five strains were 56.7%, 43.7%, and 22.5%, respectively. On comparing the average infection by the strains for the three pest species, those that stood out significantly ($P < 0.05$) were HNI at 66.7% and Hb1 at 60.4%, followed by Hb2 (34.0%), Sc (25.0%), and Sr (18.7%). The average survival rate was highest for *P. menetriesi* at 89.2%, followed by *Anomala* sp. (70.4%) and *Phyllophaga* sp. (56.2%). The total average for the last pest, by strain, presented two ranges: for the *Steinernema* strains 93.1% (Sr) to 86.8% (Sc), and for the *Heterorhabditis* strains 73.6% (Hb2), through 56.9% (Hb1) to 49.3% (HNI).

By other hand, it was demonstrated that certain developmental stages of the pests are more susceptible to these microorganisms. Hence, we evaluated the effect of the entomonematodes *Heterorhabditis* sp. (HNI; from CENICAFE) and *Steinernema feltiae* (Sf; from Villapinzón) on the mortality of different stages of these two species of white grubs: first-instar larva (L1), L2, L3 young, L3 mature, and prepupa. A concentration of 10,000 EPNs/ml was applied to larvae maintained in organic soil, under controlled laboratory conditions (24.5 °C and 70% \pm 5% RH); evaluating its effect 10 and 20 days after infection (dai). The EPN strains presented differences for *Anomala inconstans* ($P \leq 0.05$), with the highest mortality rate achieved by strain HNI at 84.7%, compared with Sf at 76.7%, for the different instars. However, L2 was the most susceptible to the

first strain (98.3%). No differences were observed between evaluation periods for this species. The highest mortality rate for *P. menetriesi* occurred 20 dai with strain HNI, again with L2 being the most susceptible stage (81.1%).

The susceptibility of white grubs to EPNs was determined as being dependent on both the species and strain of entomopathogen used—important aspects, together with knowledge of the insect's dynamics, to take into account in developing biological control programs for white grubs (Melo et al. 2007). These results are relevant, as the initial stages of white grubs take place close to the soil surface, making control, using these entomonematodes, relatively easy.

More research needs to be conducted on the defense mechanisms that pest larvae use, such as physical barriers, as in the case of *P. menetriesi* (Melolonthidae), where the cuticle is thicker than in *Cyclocephala* (Dynastinae); external melanized callosities that impede entry of parasitoids, as observed in field studies in northern Cauca (Pardo 2000); or, movement through soil to evade antagonists (M Londoño 2002, pers. comm.).

Stemborers

Known control methods were first evaluated in the 1980s, when research on the pest began. The methods that stand out are the treatment of planting stakes, and applications of the bacterium *Bacillus thuringiensis*, the fungus *Spicaria* sp., or a suspension of liquefied larvae killed by disease (probably viral). The mortality rates were as follows: 100% with the solution of macerated larvae, 99% with *B. thuringiensis*, and 88% with *Spicaria* sp. (Lohr 1983; Herrera 1999). The high mobility of the early larva-like instars of the stemborers makes them highly vulnerable and easily controlled by *B. thuringiensis*.

Because adult stemborers are difficult to kill and larvae eat inside the stems, controlling them with insecticides is not practical. Practices that will reduce pest populations are the removal and burning of infested plant parts. Only stakes that have no infestation or damage should be left to stand (Bellotti et al. 1983a).

Several natural enemies have been identified, including hymenopteran parasites and parasitoids such as *Bracon* sp., *Apanteles* sp., and *Brachymeria* sp. (Lohr 1983).

Lace bugs

At CIAT, the bug *Zelus nugax* (Hemiptera: Reduviidae) was observed to be an excellent predator of nymphs and adults of cassava lace bugs (*Vatiga* spp.), consuming, during its biological cycle, an average of 496 individuals of the pest.

Controlling lace bugs seems difficult, with few natural enemies having been found (Bellotti et al. 1999). Continuous use of insecticides is expensive and can destroy the natural enemies of other pests. Preliminary surveys and evaluations of the cassava germplasm bank held at CIAT indicate the presence of varietal resistance in the crop. Implementing such technology will, however, require considerable research (CIAT 1990).

Conclusions

In CIAT's cassava entomology program, the staff and students, both national and foreign, have carried out many tasks towards controlling the pests described above, using natural enemies. With these tools, the conditions of such an important crop can be improved for millions of people around the world.

Although the efficiency of infectivity and mortality observed in the laboratory is known to decline dramatically in the field, few studies have been applied on a field scale, particularly those seeking information on the effective application of biocontrol agents to better control subterranean insects such as white grubs. Some successful field studies include those on cassava hornworm and baculoviruses, the mealybug and *Epidinocarsis lopezi* in Africa, and the mite *M. tanajoa* and its predator *T. aripo* in Africa.

The next step is to implement these methodologies with farmers to awaken their interest in responsible environmental management and in using pesticides to a minimum.

While battles have been won, the war continues. Food production should be increased, in a scientific way, to meet requirements of the growing human society, while protecting and conserving natural resources and complying with the parameters of good agricultural practices. Agriculture should continue to perform its function as a motor of change. The "silent revolutionaries", that is, the small farmers, the authorities responsible for formulating policies, scientists, and donors have but only one option: to continue being committed to this task (WD Hopper, cited in Wigg 1994).

References

To save space, the acronym "CIAT" is used instead of "Centro Internaccional de Agricultura tropical".

Arias B; Bellotti AC. 1977. Eficiencia de *Bacillus thuringiensis* sobre el gusano cachón (*Erinnyis ello*) en yuca, en un programa de control biológico. Rev Colomb Entomol 3(3-4):93-97.

Arias B; Bellotti AC. 1985. Aspectos ecológicos y de manejo de *Cyrtomenus bergi* Froeschner, la chinche de la viruela, en el cultivo de la yuca (*Manihot esculenta* Crantz). Rev Colomb Entomol 11(2):42-46.

Akhurst RJ; Boemare NE. 1990. Biology and taxonomy of *Xenorhabdus*. In: Gaugler R; Kaya HK, eds. Entomopathogenic nematodes in biological control. CRC Press, Boca Raton, FL, USA. p 75-90.

Australian Museum. 2009. Predators, parasites and parasitoids. Sydney, Australia. (Available at <http://australianmuseum.net.au/predators-parasites-and-parasitoids>)

Banegas JA; Cave R D. 1995. Biología y diversidad de depredadores. In: Cave R, ed. Manual para la enseñanza del control biológico en América Latina, 1st ed. Zamorano Academic Press, Zamorano, Honduras. p 39-49.

Barberena MF. 1996. Capacidad parasítica de dos razas del nematodo *Heterorhabditis bacteriophora* Poinar (Rabditida: Heterorabditidae) sobre la chinche de la viruela de la yuca *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae) en condiciones de laboratorio. Thesis. Universidad del Valle, Cali, Colombia. 89 p.

Bellotti AC; Schoonhoven A van. 1978. Cassava pests and their control. CIAT, Cali, Colombia. 71 p.

Bellotti AC; Reyes JA; Varela AM. 1983a. Observaciones de los piojos harinosos de la yuca en las Américas; su biología, ecología y enemigos naturales. In: Reyes JA, ed. Yuca: Control integrado de plagas. CIAT, Cali, Colombia p 313-339.

Bellotti AC; Reyes JA; Arias B; Vargas O. 1983b. Insectos y ácaros de la yuca y su control. In: Reyes JA (ed.). Yuca: Control integrado de plagas. CIAT, Cali, Colombia p 69-94.

- Bellotti AC; Arias B; Guzmán OL. 1992. Biological control of the cassava hornworm *Erinnyis ello* (Lepidoptera: Sphingidae). Fla Entomol 75:506–515.
- Bellotti AC; Braun AR; Arias B; Castillo JA; Guerrero JM. 1994. Origin and management of Neotropical cassava arthropod pests. Afr Crop Sci J 2(4):407–417.
- Bellotti AC; Smith L; Lapointe SL. 1999. Recent advances in cassava pest management. Annu Rev Entomol 44:343–370.
- Bennett FD; Greathead PJ. 1978. Biological control of the cassava mealybug (*Phenacoccus manihoti* Matile-Ferrero): prospects and necessity. In: Brekelbaum T; Bellotti A; Lozano JC, eds. Cassava protection workshop, Cali, Colombia, 1977. CIAT, Cali, Colombia. p 181–194.
- Bento JMS; Bellotti AC; Castillo JA; de Moraes GJ; Lapointe SL; Warumby JF. 1999. Introduction of parasitoids for control of cassava mealybugs in northeastern Brazil. Bull Entomol Res 89(5):403–410.
- Bertschy C; Turlings TCL; Bellotti AC; Dorn S. 1997. Chemically-mediated attraction of three parasitoid species to mealybug-infested cassava leaves. Fla Entomol 80(3):383–395.
- Bohórquez A. 1995. Caracterización de poblaciones de *Mononychellus tanajoa* CIAT, 1982. In: Ácaros presentes en el cultivo de la yuca y su control. Guía de estudio. CIAT, Cali, Colombia. 36 p.
- Braun AR; Bellotti AC; Guerrero JM; Wilson LT. 1989. Effect of predator exclusion on cassava infested with tetranychid mites (Acari: Tetranychidae). Environ Entomol 18(4):711–714.
- Braun A; Alvarez JM; Cuéllar ME; Duque MC; Escobar JR; Franco C; Gaigl A; Guerrero JM; Lenis JI; Melo EL; Mesa NC; Zuñiga R. 1993. Inventario de ácaros fitófagos y sus enemigos naturales en el cultivo de la yuca en Ecuador. In: Braun AR, ed. Bases fundamentales para la investigación sobre los ácaros plaga y sus enemigos naturales en el Ecuador. Working document no. 126. CIAT, Cali, Colombia p 1–51.
- Byrne DH; Bellotti AC; Guerrero JM. 1983. The cassava mites. Trop Pest Manage 29(4):378–394.
- Caicedo AM. 1993. Evaluación del parasitismo del nematodo entomógeno *Steinernema carpocapsae* Weiser (Rhabditida: Steinernematidae) y reconocimiento de nemátodos nativos para el control de *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae). Agronomy thesis. Faculty of Agricultural Sciences, Universidad Nacional de Colombia–Palmira, Colombia. 101 p.
- Caicedo AM; Bellotti AC. 1994. Evaluación del potencial del nematodo entomógeno *Steinernema carpocapsae* Weiser (Rhabditida: Steinernematidae) para el control de *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae) en condiciones de laboratorio. Rev Colomb Entomol 20(4):241–246.
- Caicedo AM; Bellotti AC. 1996. Reconocimiento de nematodos entomopatógenos nativos asociados con *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae) en ocho localidades de Colombia. Rev Colomb Entomol 22(1):19–24.
- Carter SE, Fresco LO, Jones PG; Fairbairn JN. 1992. An atlas of cassava in Africa. Historical, agroecological and demographic aspects of crop distribution. CIAT, Cali, Colombia. 85 p.
- Castañón PO; Bellotti AC; Vargas O. 1985. Efecto del HCN y de los cultivos intercalados en la 'chinche de la viruela' (*Cyrtomenus bergi* Froeschner) y en el daño que causa al cultivo de la yuca. Rev Colomb Entomol 11(2):24–26.
- Castillo P; Acosta N; Ciliézar A. 1995. Control microbiológico de plagas artrópodas. In: Cave RD, ed. Manual para la enseñanza del control biológico en América Latina. Zamorano Academic Press, Zamorano, Honduras. p 51–72.
- Castillo J. 1996. Moscas blancas (Homoptera: Aleyrodidae) y sus enemigos naturales sobre cultivos de yuca (*Manihot esculenta* Crantz) en Colombia. MSc thesis. Universidad del Valle, Cali, Colombia. 173 p.
- Cave RD. 1995a. Perspectivas del control biológico. In: Cave RD, Ed. Manual para la enseñanza del control biológico en América Latina. Zamorano Academic Press, Zamorano, Honduras. p 7–9.
- Cave RD. 1995b. La taxonomía y sistemática en el control biológico. In: Cave RD, ed. Manual para la enseñanza del control biológico en América Latina. Zamorano Academic Press, Zamorano, Honduras. p 17–21.

- Cave RD. 1995c. Características deseables de un buen enemigo natural para el control de plagas. In: Cave RD, ed. Manual para la enseñanza del control biológico en América Latina. Zamorano. Academic Press, Zamorano, Honduras. p 23–25.
- CIAT. 1982. Ácaros presentes en el cultivo de la yuca y su control. Guía de estudio. Cali, Colombia. 36 p.
- CIAT. 1989. Manejo integrado de *Erinnyis ello* (L.), gusano cachón de la yuca. Guía de estudio para ser usada como complemento de la unidad audiotutorial del mismo tema. Cali, Colombia. 62 p.
- CIAT. 1990. Biological control of cassava green mite. In: Cassava Program, Annual report. Cali, Colombia. p 129–179.
- CIAT. 1995. Annual report, Cassava Program, 1994. Cali, Colombia. p 144–163.
- CIAT. 1999. Annual report, Integrated Pest and Disease Management in Major Agroecosystems. Cali, Colombia. 136 p.
- CIAT. 2003. Soil pest-cassava and others crops. In: Annual Report. Integrated Pest and Disease Management in Major Agroecosystems. Cali, Colombia. p 53–70.
- DeBach P. 1975. El alcance del control biológico. In: Control biológico de las plagas de insectos y malas hierbas. Compañía Editorial Continental S.A., Mexico. 949 p.
- DeBach P. 1977. Ecología del control biológico. In: Lucha biológica contra los enemigos de las plantas. Ediciones Mundi-Prensa, Madrid, Spain. 395 p.
- Delalibera Jr I; Sosa-Gómez DR; Moraes GJ; de Alencar JA; Farias-Araujo W. 1992. Infection of *Mononychellus tanajoa* (Acari: Tetranychidae) by the fungus *Neozygites* sp. (Entomophthorales) in Northeastern Brazil. Fla Entomol 75(1):145–147.
- Díaz FA; Hanson P. 1995. Biología y diversidad de parasitoides. In: Cave R, ed. Manual para la enseñanza del control biológico en América Latina. Zamorano Academic Press, Zamorano, Honduras. p 27–37.
- Evans GA; Castillo JA. 1998. Parasites of *Aleurotrachelus socialis* (Homoptera: Aleyrodidae) from Colombia including descriptions of two new species (Hymenoptera: Aphelinidae: Platygasteridae). Fla Entomol 81(2):171–178.
- Ferron P. 1985. Fungal control. In: Kerkut GA; Gilbert LI, eds. Comprehensive insect physiology, biochemistry, and pharmacology, vol 12: Insect control. Pergamon Press, New York. p 313–346. (13 vols.)
- Gaugler R; Kaya HK, eds. 1990. Entomopathogenic nematodes in biological control. CRC Press, Boca Raton, FL, USA. 365 p.
- Hajek AE, St Leger RJ. 1994. Interactions between fungal pathogens and insect host. Annu Rev Entomol 39:293–322.
- Herren HR; Neuenschwander P. 1991. Biological control of cassava pests in Africa. Annu Rev Entomol 36:257–283.
- Herrera CJ. 1999. Manejo integrado de plagas en el cultivo de la yuca. In: Seminar-workshop “Hacia una producción bioracional de la yuca”, held at Pivijay, El Carmen de Bolívar, Feb 1999. PMD; IICA; BIOCARIBE S.A., Medellín, Colombia. 45 p.
- Jaramillo J. 2004. Control of subterranean burrower bug *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae) using entomopathogenic fungi (Deuteromycotina: Hyphomycetes). MSc thesis in Horticulture. University of Hannover, Germany. 24 p.
- Laberry R. 1997. La aplicación de un programa MIP en producción industrial de yuca. In: Proc. Congreso de Fitopatología, Biodiversidad y Micorrizas, held at CIAT, Cali. Asociación Colombiana de Fitopatología y Ciencias Afines (ASCOLFI), Palmira, Colombia. p 136–137.
- Lacey LA; Brooks WM. 1997. Initial handling and diagnosis of diseased insects. In: Lacey LA, ed. Manual of techniques in insect pathology. Biological Techniques Series. Academic Press, NY. p 1–15.
- Leuschner K; Nwanze K. 1978. Preliminary observations of the mealybug (Hemiptera: Pseudococcidae) in Zaire. In: Brekelbaum T; Bellotti AC; Lozano JC, eds. Cassava protection workshop, Cali, Colombia, 1977. CIAT, Cali, Colombia. p 195–202.

- Lohr B. 1983. Biología, ecología, daño económico y control de *Chilomima clarkiei* (Amsel) (Lepidoptera, Pyralidae) barrenador de la yuca. In: Reyes JA, ed. Yuca: Control integrado de plagas. CIAT, Cali, Colombia. p 159–161.
- Melo EL. 2000. El potencial de control biológico en el manejo de plagas. In: Symposium “Avances en el Manejo de Plagas”. Proc XXVII Congress of SOCOLEN, held in Medellín, July 2000. Sociedad Colombiana de Entomología (SOCOLEN), Bogotá, Colombia. p 219–242.
- Melo EL; Ortega-Ojeda CA; Gaigl A. 2007. Efecto de nematodos sobre larvas de *Phyllophaga menetriesi* y *Anomala inconstans* (Coleoptera: Melolonthidae). Rev Colomb Entomol 33(1):21–26.
- Melo EL; Ortega-Ojeda CA; Susurluk A; Gaigl A; Bellotti AC. 2009. Poblaciones nativas de nematodos entomopatógenos (Rhabditida) en cuatro departamentos de Colombia. Rev Colomb Entomol 35(1):95–100.
- Moraes GJ; Delalibera Júnior I. 1992. Specificity of a strain of *Neozygites* sp. (Zygomycetes: Entomophthorales) to *Mononychellus tanajoa* (Acari: Tetranychidae). Exp Appl Acarol 14:89–94.
- Múnera DF; De los Ríos J; Bellotti AC. 1999. Patogenicidad sobre *E. ello* (Lepidoptera: Sphingidae) en condiciones de laboratorio por hongos entomopatógenos recolectados en cultivos comerciales de yuca, *Manihot esculenta*, en el Valle del Cauca, Colombia. Rev Colomb Entomol 25(3–4):161–167.
- Nyiira ZM. 1972. Cassava: investigations 1972–1973. In: Kawanda Research Station, Uganda: Annual Report (Part 2), Entomology Section. Kampala, Uganda. 6 p.
- Odongo B; Kumar R; Odindo MO; Brownbridge M. 1988. The effectiveness of entomogenous fungus *Hirsutella* sp. (fungi imperfecti) in controlling cassava green mite, *Mononychellus tanajoa* (Acari: Tetranychidae). In: Proc 8th Symposium of the International Society for Tropical Root Crops, Bangkok, Thailand. 354 p.
- Ortega GA. 1999. Determinación de la efectividad de *Encarsia hispida* DeSantis (Hymenoptera: Aphelinidae) como parasitoide de la ‘mosca blanca de la yuca’, *Aleurotrachelus socialis* Endar (Homoptera: Aleyrodidae), bajo condiciones de invernadero. BSc thesis. Universidad Nacional de Colombia–Palmira, Colombia. 57 p.
- Pardo LC. 2000. Avances en el estudio de chisas rizófagas (Coleoptera: Melolonthidae) en Colombia, observaciones sobre los complejos regionales y nuevos patrones morfológicos de larvas. In: Symposium “Avances en el Manejo de Plagas”. Proc XXVII Congress of SOCOLEN, held in Medellín, Sociedad Colombiana de Entomología (SOCOLEN). Bogotá, Colombia. p 285–306.
- Sáenz A. 1999. Los nematodos entomopatógenos: Una alternativa del control biológico. In: Proc XXVI Congress of SOCOLEN. Sociedad Colombiana de Entomología (SOCOLEN), Bogotá, Colombia. p 82–97.
- Sánchez D. 1996. Patogenicidad de hongos Hyphomycetes sobre *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae), chinche subterránea de la yuca, en condiciones de laboratorio. Thesis. Faculty of Agricultural Sciences, Universidad Nacional de Colombia–Palmira, Colombia. 100 p.
- Sánchez D; Bellotti AC. 1997a. Patogenicidad de hongos Hyphomycetes sobre *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae), chinche subterránea de la yuca. Rev Colomb Entomol 23(1/2):31–37.
- Sánchez D; Bellotti AC. 1997b. Evaluación de la patogenicidad de hongos Hyphomycetes sobre mosca blanca de la yuca *A. socialis*. Report on the Cooperative Agreement CIAT–Colciencias, Programa BID para jóvenes investigadores. CIAT, Cali, Colombia. 20 p.
- Schmitt AT. 1988. Uso de *Baculovirus erinnyis* para control biológico del gusano cachón de la yuca. Yuca Bol Inf 121:1–4.
- Stock SP. 1998. Sistemática y biología de nematodos parásitos y asociados a insectos de importancia económica. In: International Entomopathogenic Nematodes and Insect Pathology Courses. Universidad Nacional del Litoral (UNL). Editorial Esperanza, Santa Fé, Argentina. 106 p.
- Tanada Y; Kaya HK. 1993. Insect pathology. Academic Press, San Diego, CA, USA. p 318–387.

- Trujillo HE; Arias B; Guerrero JM; Bellotti AC. 1999. Estudio del complejo y distribución de especies de parasitoides de mosca blanca en el cultivo de la yuca (*Manihot esculenta* Crantz) en diversas zonas de Colombia. In: Abstracts XXVI Congress of SOCOLEN, held in Bogotá, DC, Colombia, July 1999. Sociedad Colombiana de Entomología (SOCOLEN), Bogotá, Colombia. 123 p.
- van Driesche RG; Castillo JA; Bellotti AC. 1988. Field placement of mealybug-infested potted cassava plants for the study of parasitism of *Phenacoccus herreni*. Entomol Exp Appl 46:117–123.
- van Driesche RG; Bellotti AC; Castillo JA; Herrera CJ. 1990. Estimating total losses from parasitoids for a field population of a continuously breeding insect, cassava mealybug, *Phenacoccus herreni* (Hemiptera: Pseudococcidae) in Colombia, S.A. Fla Entomol 73:133–143.
- Wigg D. 1994. Los revolucionarios silenciosos. Una reseña de la campaña contra el hambre que llevan a cabo los científicos agrícolas. World Bank, Washington, DC, USA. p 1–11.
- Yaninek JS; Bellotti AC. 1987. Exploration for natural enemies of cassava green mite based on agrometeorological criteria. In: Rijks D; Mathys G, eds. Seminar on agrometeorology and crop protection in the lowland humid and subhumid tropics, held in Cotonou, Benin, July 1986. World Meteorological Organization, Geneva, Switzerland. p 69–75.
- Yaninek JS; Herren HR. 1988. Introduction and spread of the cassava green mite, *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae), an exotic pest in Africa, and the search for appropriate control methods: a review. Bull Entomol Res 78:1–13.
- Yaninek JS; Mégev B; De Moraes GJ.; Bakker F; Braun A. 1991. Establishment of the Neotropical predator *Amblyseius idaeus* (Acari: Phytoseiidae) in Benin, West Africa. Biocontrol Sci Technol 1(4):323–330.
- Yaninek JS; Onzo A; Ojo JB. 1993. Continent-wide releases of Neotropical phytoseiids against the exotic cassava green mite in Africa. Exp Appl Acarol 17(1/2):145–160.
- Yaninek JS; Saizonou S; Onzo A; Zannou I; Gnanvossou D. 1996. Seasonal and habitat variability in the fungal pathogens, *Neozygites c.f. floridana* and *Hirsutella thompsonii*, associated with cassava mites in Benin, West Africa. Biocontrol Sci Technol 6(1):23–33.